

CLAIMS

1. A method of introducing one or more exogenous substances into an immune cell, the method comprising,
 - 5 providing a membrane having a plurality of pores configured to allow the immune cell to pass through, wherein the plurality of pores has an average pore diameter which is no less than an average diameter of the immune cell nucleus and no more than an average diameter of the immune cell,
 - passing a fluid containing the immune cell and the one or more exogenous
10 substances through the plurality of pores to induce a mechanical stress to the immune cell and facilitate introduction of the one or more exogenous substances into the immune cell, and
 - recirculating the fluid containing the immune cell and the one or more exogenous substances such that the same immune cell passes through the
15 membrane more than once;
 - wherein a specific flow rate for passing the fluid containing the immune cell and the one or more exogenous substances through the plurality of pores is from 10 nL/s to 1000 nL/s.
- 20 2. The method according to claim 1, further comprising, prior to the step of passing the fluid, activating the immune cell from an inactivated to an activated state by exposing the immune cell to one or more activation agents.
3. The method according to claim 1 or 2, wherein the method is devoid
25 of introducing one or more conditioning substances into the fluid after the activating step.
4. The method according to any one of claims 1 to 3, wherein the immune cell has not been pretreated to alter cell membrane pliability, prior to
30 passing through the membrane.

5. The method according to any one of claims 1 to 4, further comprising, prior to passing the immune cell through the membrane, seeding the immune cell into the fluid at a density of no less than 1×10^5 cells/ml and adding the one or more exogenous substances into the fluid.

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6. The method according to any one of claims 1 to 5, wherein the fluid containing the immune cell and the one or more exogenous substances has a volume of at least 20 mL.

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7. The method according to any one of claims 1 to 6, wherein the fluid containing the immune cell and the one or more exogenous substances is substantially free of serum when the fluid is passed through the plurality of pores.

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8. The method according to any one of claims 1 to 7, further comprising providing a time interval of at least 5 minutes to rest the immune cell between any two consecutive passes through the membrane.

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9. The method according to any one of claims 1 to 8, wherein the immune cell is a T cell.

10. The method according to claim 9, wherein the T cell transfected with the one or more exogenous substances is to be used for immunotherapy.

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11. The method according to any one of claims 1 to 10, wherein the one or more exogenous substances comprise a nucleic acid configured for expressing one or more exogenous genes for a therapeutic application.

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12. The method according to any one of claims 1 to 11, wherein, for each pass through the plurality of pores, the number of viable immune cells recovered after passing through the plurality of pores is at least 30% of the number of viable immune cells prior to passing through the plurality of pores.

13. The method according to any one of claims 1 to 12, wherein, for each pass through the plurality of pores, the number of immune cells transfected with the one or more exogenous substances is at least 10% of the number of untransfected immune cells prior to passing through the plurality of pores.

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14. The method according to any one of claims 1 to 13, wherein the number of immune cells transfected with the one or more exogenous substances increases with each additional pass across the membrane.

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15. A system for introducing one or more exogenous substances into an immune cell, the system comprising,

a membrane having a plurality of pores configured to allow the immune cell to pass through, wherein the plurality of pores has an average pore diameter which is no less than an average diameter of the immune cell nucleus and no
15 more than an average diameter of the immune cell, and

a pump configured to pass a fluid containing the immune cell and the one or more exogenous substances through the plurality of pores to induce a mechanical stress to the immune cell and facilitate introduction of the one or more exogenous substances into the immune cell,

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wherein the pump is further configured to recirculate the fluid containing the immune cell and the one or more exogenous substances such that the same immune cell passes through the membrane more than once, and

wherein the pump is further configured to pass the fluid containing the immune cell and the one or more exogenous substances through the plurality of
25 pores at a specific flow rate of from 10 nL/s to 1000 nL/s.

16. The system according to claim 15, further comprising a vessel arranged to form an enclosed system such that the membrane is housed within the enclosed system.